1971

Effect of Splenectomy on Relapse of Hemal Parasites in Pigeons

Phillip James Sprino
Eastern Illinois University

PAPER CERTIFICATE

TO: Graduate Degree Candidates who have written formal theses.

SUBJECT: Permission to reproduce theses.

The University Library is receiving a number of requests from other institutions asking permission to reproduce dissertations for inclusion in their library holdings. Although no copyright laws are involved, we feel that professional courtesy demands that permission be obtained from the author before we allow theses to be copied.

Please sign one of the following statements.

Booth Library of Eastern Illinois University has my permission to lend my thesis to a reputable college or university for the purpose of copying it for inclusion in that institution's library or research holdings.

June 1, 1971
Date

I respectfully request Booth Library of Eastern Illinois University not allow my thesis be reproduced because _____

_____

_____

_____ _____

Date Author

EFFECT OF SPLENECTOMY ON RELAPSE OF HEMAL PARASITES IN PIGEONS

(TITLE)

BY

Phillip James Sprino
B.S. Eastern Illinois University, 1968

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science in Zoology

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1971
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
THIS PART OF THE GRADUATE DEGREE CITED ABOVE

1 June 1971
DATE

1 June 1971
DATE

The undersigned, appointed by the Head of the Department of Zoology, have examined a thesis entitled

EFFECT OF SPLENECTOMY ON RELAPSE
OF HEMAL PARASITES IN PIGEONS

Presented by

PHILLIP JAMES SPRINO

a candidate for the degree of M.S. in Zoology
and hereby certify that in their opinion it is acceptable.

_____

_____

_____

_____

_____

_____

EXAMINATION CERTIFICATE

Master's Degree Certificate
for
Comprehensive Examination

I certify that _____ has successfully passed

The examining committee consisted of:

Signatures of the Committee

May 17, 1971
DATE

ACKNOWLEDGMENTS

I wish to extend a special thanks to Dr. B. T. Ridgeway who suggested the problem and whose direction and reassurance throughout the investigation were invaluable. Appreciation is expressed to Dr. G. T. Riegel, Dr. V. B. Kniskern, Dr. R. D. Andrews, and Dr. H. C. Rawls for aid and suggestions offered from time to time. I am grateful to Mr. Donald D. Sundheim and Mr. Joseph T. Babicki for translation of papers. Thanks are due to Mr. Frank H. Hedges for photomicrography. Finally, I would like to express my gratitude to many members of the Department of Zoology, both faculty and students, who helped in numerous ways during the course of the study.

TABLE OF CONTENTS

LIST OF TABLES, PLATES AND FIGURES

Tables

Plates

Figures

Page

INTRODUCTION

The sporozoan parasites Haemoproteus columbae and Plasmodium relictum are known to have a latent phase in pigeons (Columba livia). Relapse of latent infections is a striking feature of many malarial infections of mammals and of some haemosporidian parasites of birds (such as Leucocytozoon). This phenomenon also occurs in latent infections of H. columbae of pigeons due to a decline of host immune mechanisms. On the other hand, true relapses appear to be absent, under natural situations, in avian Plasmodium infections (Garnham, 1966). However, relapse and recrudescense have been reported to take place under experimental situations subsequent to spleen removal, for Plasmodium cathemerium in canaries (Causey, 1939), P. gallinaceum in chicks (Brumpt, 1936; Terzian, 1946; Taliaferro, 1948; Corradetti, 1955; El-Nahal, 1966), P. rouxi in canaries (Corradetti and Verolini, 1958), P. juxtanucleare in chicks (Al-Dabagh, 1960), P. relictum in pigeons and P. lophurae in chicks (Longenecker, 1968). An extension of the conclusions drawn from the above investigations is that splenectomy affects the state of the host-parasite relationship. This is true for malarial infections as well as other protozoan infections.

Little work has been done on the effect of splenectomy on the relation between pigeon host, and malarial parasites. If splenectomy of pigeons harbouring latent infections of malarial organisms can produce relapse in a significant number of cases, then it is possible that this procedure may be used as a method of detecting latent infections in natural populations of these birds.

Fox (1968), using conventional blood smear techniques in sampling a large population (approximately 2,000 birds) of pigeons occurring in Central Illinois, determined that 27.8 per cent of the birds sampled were infected with Haemoproteus columbae, and 2.8 per cent were infected with Plasmodium relictum matutinum. The present investigation was undertaken to compare the incidence of infection with H. columbae and P. relictum demonstrated by standard blood smear techniques (Fox, 1968), with a procedure using splenectomy to expose these and other hemal parasites. A secondary objective was to describe an adequate method of splenectomy for the pigeon (Columba livia).

LITERATURE REVIEW

Splenectomy in Induced Relapse

Relapse of avian malaria subsequent to splenectomy has been reported for several species of birds. Causey (1939) removed the spleen from canaries known to have been previously infected with Plasmodium cathemerium. Three birds were splenectomized 6, 12, and 21 days after the last parasites were observed in the blood. The most severe relapses occurred in the canary which was splenectomized on the sixth day of the latent phase of infection. The relapses were progressively less severe following spleen removal on the twelfth and twenty-first day of latency. The bird splenectomized on the twenty-first day of latency gradually reached a peak infection and made a rapid recovery. The other two canaries more quickly reached a peak of infection and died presumably from the malarial parasites.

Brumpt (1936) reported a relapse of bird malaria by utilizing spleen removal. He obtained a relapse of Plasmodium gallinaceum in chicks but gave no further information.

Removal of the spleen from chicks harbouring latent infections of P. gallinaceum produced increased parasitemias of varying degrees, according to work by Terzian (1946). It was noted that excessive hemorrhage and simple laparotomy, without removal of any tissues, upon chicks with latent infections did not induce relapse; indicating that simple injury to the host does not seriously affect the immune mechanisms. Terzian concluded that although the spleen was specifically concerned with maintaining an established immunity to avain malaria, the characteristics of the parasite

largely determine the importance of the spleen to this immunity during latency.

Induced relapse of Plasmodium gallinaceum in chicks has been reported by Taliaferro (1948) and Taliaferro and Kelsey (1948). Spleen removal was found to decrease acquired immunity without noticeably affecting innate immunity against infection of P. gallinaceum. The decrease in acquired immunity was manifested in a higher parasitemia in splenectomized birds as well as a greater mortality rate of chicks in both investigations above. However, no alteration occurred on the course of parasitemia or on the mortality rate of individuals splenectomized prior to infection.

Corradetti (1955) made studies of comparative pathology and immunity in Plasmodium infections of chicks. Results of this investigation demonstrated relapse of avian malaria. A surge in P. gallinaceum in birds harbouring latent infections occurred subsequent to splenectomy.

The action of splenectomy on the course of infection of Plasmodium rouxi in canaries was investigated by Corradetti and Verolini (1958). From the results obtained, it was evident that splenectomy carried out before injections of P. rouxi or during the infection, produced modifications in the course of this infection when compared with non-splenectomized birds. Incubation time and duration of host survival were less in canaries splenectomized prior to infection, as compared with non-splenectomized individuals. Corradetti and Verolini concluded that splenectomy brought about a more active multiplication of P. rouxi in the vertebrate host. This was indicated not only by a shorter incubation time in birds previously splenectomized, but also by the progressive numerical increase of parasites until death. This was not found to be the case for non-splenectomized canaries.

Splenectomy has also resulted in increased parasitemias and mortality rates in ducklings infected with Plasmodium elongatum (Manwell, et al., 1957).

Spleen removal on birds younger than three weeks resulted in more severe and prolonged parasitemias. The operation on older ducks, prior to infection, almost completely eliminated their age resistance to P. elongatum, and the resulting infection was comparable to that in ducklings. However, when spleen removal occurred after development of acquired immunity, this immunity is little affected.

It has been reported (Al-Dabagh, 1960) that splenectomy causes a marked recrudescence in chicks infected with Plasmodium juxtanucleare if performed at the chronic stage of infection. In this study, all of thirteen splenectomized birds showed a marked recrudescence in two or three days after the operation. The parasitemia in these individuals rose to a level which had never been observed in any of the non-splenectomized chicks (up to 65 per cent of the R.B.C.'s were infected during the recrudescence). The mortality rate was much higher for splenectomized birds (54 per cent) as compared with non-splenectomized chicks.

El-Nahal (1966) studied the effects of splenectomy on infections of P. gallinaceum in chickens. Six-week old chicks were inoculated, intraperitoneally, with blood from a fowl showing high parasitemia. Splenectomy was performed seventy-eight days later. The first parasitized blood cell was observed on the fourth day after inoculation and the parasite number reached a maximum on the eighth day of infection. By the twenty-fifth day, only a few parasites could be detected. Eventually, no parasites could be observed and on the seventy-ninth day splenectomy was performed. Three days after spleen removal, parasitized erythrocytes once again began to appear. The infection increased rapidly and reached a crisis ten days after the operation. Following the crisis, only a few parasites were observed by the twentieth day post-splenectomy.

Relapse of avian malaria has also been observed by Becker and Farmer, according to Longenecker (1968). A relapse of Plasmodium relictum in a pigeon was noticed, by these two men, as a result of splenectomy.

Longenecker (1968) investigated the effect of spleen removal on Plasmodium lophurae in chicks at various times during the infection. According to Garnham (1966), young chicks are susceptible to both sporozoite and blood forms of P. lophurae; they develop an acute attack, which lasts for one or two weeks, and then declines rapidly. The maximum parasitemia is not much more than 10 per cent and is much lower in adult birds. Longenecker found that the effect of splenectomy at eighteen days of age on a Plasmodium infection occurring approximately three weeks later, resulted in a surge of up to 55 per cent parasitemia. This sharp increase in parasitemia took place within seven days after infection. As a matter of fact, all of the chicks referred to above were dead by the seventh day. The effect of splenectomy on the fifth day after Plasmodium infection resulted in an immediate recrudescence of the parasitemia in a second experiment. Splenectomy was performed when the parasitemia was approximately 2 per cent. Of the five birds in this second group, three died with parasitemias of about 60 per cent by the eighth day post-infection, but two individuals recovered by the tenth day. The effect of splenectomy on reinfection, after recovery from initial infection, comprised a third line of investigation by Longenecker. He found that chicks splenectomized after recovery from the primary infection demonstrated no ability to successfully resist reinfection. All three birds of this group showed high parasitemias of P. lophurae and died by day five of the reinfection.

There have been many contradictory results concerning the effect of splenectomy on avian malarial infections. It has been reported that splenectomy prior to infection does not modify the course of parasitemia or

or mortality rate for P. circumflexum in canaries (Herman and Goldfarb, 1939), P. cathemerium in canaries (Causey, 1939), P. gallinaceum in chicks (Terzian, 1946; Taliaferro, 1948), P. juxtanucleare in chicks (Al-Dabagh, 1960), and P. lophurae in chicks as reported by Terzian (1946).

Experimental Investigations on Induced Relapse

Various experimental methods, other than splenectomy, have been utilized in attempts to induce relapse of avian malaria. Moldovan was reported to have produced experimental relapse of avian malaria by injecting foreign blood into the host (Hewitt, 1940). Whitmore (1922) claims to have produced relapse in birds, with latent infections of P. relictum, by exposing them to an unfiltered light of a quartz mercury vapor lamp for two hours at a distance of two feet. According to Ben-Harel (1923), malarial relapse was reported for birds subjected to radiation with ultraviolet light. Hewitt (1940) was convinced that X rays may produce relapse in avian malaria. Blockade of the lymphoid-macrophage system has been mentioned by Garnham (1963) as a means of provoking relapse in avian malaria. This may be accomplished by blockading the system with India ink or other substances including foreign red blood cells. Bursectomy also has been categorized as a form of immunosuppression by Abramoff and La Via (1970). The bursa of Fabricius is a lymphoid structure found in birds and has been the object of considerable interest in recent years, because it has been found to be an important part of the immune mechanism.

Spleen Function

The spleen functions as an erythropoietic organ during embryonic development. Erythropoiesis regularly occurs in the embryonic mammalian spleen (Thiel and Downey, 1921) and is presumed to also take place in birds. Specific mesenchymal cells in the spleen give rise to hemocytoblasts which in turn produce erythroblasts. This action begins by the fifth month in humans

and virtually ends by the sixth month. However, the spleen retains the ability to produce red blood cells, as may be seen with myeloproliferative disorders occurring in mammals (Blaustein, 1963). On the other hand, the spleen of the adult carrier-pigeon has shown no evidence of erythropoiesis, according to Toryu (1930).

There are three ways in which the spleen might regulate erythropoiesis while not directly involved as a production center (Crosby, 1959). (1) The spleen might increase or decrease the enzymatic synthesis of haemoglobin. (2) The spleen may cause an increase or decrease in the number of erythropoietic units in the bone marrow. (3) The spleen may determine the age at which young blood cells are released from the bone marrow. Removal of the spleen appears to permit earlier release of the red cells. Blaustein (1963) stated that there was a great deal of conjecture concerning the relationship between the spleen and bone marrow erythropoiesis and that the above three postulates were not conclusively supported. According to Blaustein, the relationships that have been verified by experimental evidence include (1) the effect of the spleen in maturation of the erythrocyte surface; (2) the culling effect of the spleen; (3) the function of the spleen in iron metabolism; and (4) the pitting function of the spleen.

Crosby (1952) reported that a splenic effect upon maturation of the red cell's surface can be demonstrated by splenectomy. As reticulocytes become mature, they undergo certain changes in addition to losing their reticulum. These cells shrink in volume and therefore in diameter as well. Certain changes in the surface of the cell also take place as maturation progresses. The somewhat "sticky" reticulocyte loses this characteristic as it becomes mature. However, splenectomy does not allow the red cell to mature normally and this observation implicates the spleen, in some way, with maturation of

the red cell surface. Miller et al. (1942) demonstrated that loss of the spleen does not increase surface area of the red cells already present, but by the process of attrition and replacement a new population of thin cells appear. The reticulocytes of this new population mature as they did in the presence of the spleen, but do not lose as much surface area and surface lipids. Therefore, the spleen is apparently involved with normal maturation of the red cell surface. Surface "stickiness" is known to determine, to some extent, the time when young cells may be released from the bone marrow. Since reticulocytes are sticky, Blaustein (1963) states that it is conceivable that the effect of the spleen on the red cell surface may influence the age when reticulocytes enter circulation.

The culling function of the spleen describes the ability of this organ to inspect the passing red blood cells and to remove from circulation those which do not fulfill certain minimum requirements (Crosby, 1959). About 10 per cent of the red cells produced in the bone marrow are defective in some way and are removed from circulation as they pass through the spleen on the first circuit through the blood stream. The spleen accomplishes its culling function by phagocytes present in the red pulp and in the walls of the splenic sinusoids.

The connection between the spleen and iron metabolism has been known for some time. According to Krumbhaar (1926), the richness of the spleen in iron and blood pigment was noticed as early as 1854 by Henry Gray. Crosby (1959) described the function of the spleen in iron metabolism and related this to the destruction of red cells. He stated that haemoglobin from the red cells is broken down and the iron is returned to erythroblasts for reuse. The spleen's reticulo-endothelial cells perform their function by phagocytosis of the red blood cell, retaining the iron and releasing the bilirubin, and by athrocytosis of colloidal iron transported by the blood plasma (Blaustein, 1963).

Barcroft (1925) demonstrated that the spleen of mammals serves as a reservoir of erythrocytes and in times of emergency expels the reserve cells into the general circulation. Crosby (1959) referred to the reservoir function of the spleen as the sequestration of red cells in the pulp and splenic sinuses. He mentioned that the spleens of certain animals are highly expansile. These animals are able to remove large proportions of red cells from active circulation, holding them perhaps for the time an emergency would arise. However, the reservoir function was not found to be an important role of the human spleen under normal situations (Ebert, 1941). The small reserve that does occur in the human spleen may be mobilized by exercise or any form of stress which results in a lowered tissue oxygen level. Harmon et al. (1932) concluded that the spleen in chickens, as in mammals, presumably acted as a reservoir and could expel red blood cells into the general circulation in emergencies. Sturkie (1965) refuted the reservoir function of the spleen as occurring in the domestic fowl. He further stated that the spleen of birds has a thin capsule with few muscle fibers, no true trabeculae, and is capable of only a slight contraction.

The spleen also functions to dispose of worn-out blood cells. Rous and Robertson (1917) suggested that worn-out cells were destroyed by fragmentation in the blood stream and the fragments were then collected in the spleen. Young red blood cells enter the circulation with healthy surfaces, which are able to escape challenge by the spleen's inspection mechanism. Eventually the erythrocyte surface becomes damaged, but enzyme systems within the cell are responsible for early repair. This enzyme system gradually wears down and the enzymatic ability to repair the stroma wanes (Allison and Bush, 1955). The stroma apparently relaxes, resulting in an increase in surface area. The cell becomes mechanically fragile according to Singer

and Weisz (1945), and therefore susceptible to fragmentation. At some point in its journey through the spleen, the erythrocyte becomes attractive to phagocytes. It is believed by some investigators that destruction of red blood cells is not totally the responsibility of the phagocyte, but that there is a physiologic hemolysin acting upon the erythrocyte surface (Blaustein, 1963).

According to Bloom and Fawcett (1962), the spleen possesses a combination of phagocytic, cytopoietic, and antibody-forming capabilities which are of importance in immunity to organisms or antigens which enter the blood. Lymphocytes are produced specifically in splenic nodules of the white pulp. From the white pulp, they migrate to the red pulp where some of them may become monocytes. Blaustein (1963) states that the lymphocyte is responsible for antibody production but that the phase in which this is demonstrable is in the preplasma cell transition stage and finally, to a lesser degree, in the plasma cell itself. The transformation of the lymphocytes to plasma cells has been described by Jordan (1954). Fagraeus (1948) demonstrated that by injecting antigen intravenously a great increase in the number of plasma cells was found in the spleen. This activity was accompanied by a simultaneous rise in circulating antibodies. Fagraeus noticed that antibody production reached a maximum when numerous immature plasma cells were present.

Techniques of Avian Splenectomy

Avian splenectomy procedures have been developed as the result of a number of scientific and practical endeavors. Investigations involving splenectomy in domestic chicks or chickens have been performed by many researchers (Brúmpt, 1936; Herman and Goldfarb, 1939; Terzian, 1946;

Taliaferro, 1948; Al-Dabagh, 1960; El-Nahal, 1966). Spleen removal in canaries has been successfully performed by Causey (1930) and also Corradetti and Verolini (1958). Splenectomy in ducks has been reported by Manwell et al. (1957). Extirpation of the spleen has been successfully performed for pigeons, according to Toryu (1930) and Jordan and Robeson (1942).

There were certain basic aspects of surgical technique that were common to most of the avian splenectomies referred to above. Some sort of preoperative preparation was normally included in surgical operations. Al-Dabagh (1960) and Taliaferro (1948) starved their experimental birds for twelve and twenty-four hours respectively, in preparation for splenectomy. As a general procedure, the feathers were plucked around the area in which laparotomy was to be made. After the feathers were removed, the area was usually sterilized with alcohol. Al-Dabagh (1960) was the most cautious of all of the above workers, with respect to aseptic conditions. After he had plucked the feathers, the area was shaved and then sterilized with iodine tincture and methylated alcohol. Toryu (1930) mentions the use of clean or sterile instruments. Taliaferro (1948) stated that only minimal sterile precautions were needed to perform splenectomies, such as swabbing the area of incision with 70 per cent alcohol. Herman and Goldfarb (1939) noted that aseptic conditions were found to be unnecessary with chicks.

Differences in administration of anesthesia was limited and varied primarily in concentration. Larger and older birds required higher concentrations or larger quantities of the same concentrations of a particular anesthetic. When anesthesia was incorporated into the technique, nembutal and ether were frequent choices. Nembutal was used by Causey (1939), Terzian (1946), and Manwell et al. (1957), while Toryu (1930), Herman and Goldfarb (1939) and Taliaferro (1948) utilized ether. Al-Dabagh (1960) demonstrated

somewhat of an innovation in that he employed nembutal as the basic anesthetic, but completed anesthesia with small amounts of ether applied to the nostrils of the chick. Brumpt (1936), Jordan and Robeson (1942) and El-Nahal (1966) offered no information concerning anesthesia in their experiments. A commercial anesthetic, called Equithesin, has been adapted for use in avian surgery. This anesthetic contains chloral hydrate, pentobarbital, and magnesium sulfate in aqueus solution of propylene glycol with alcohol, and has proven to be more suitable for bird surgery than nembutal or ether. Gandal (1956) has determined experimentally the proper dosages of Equithesin to achieve the desired level of anesthesia in such common laboratory birds as chickens, canaries, parakeets and pigeons.

Various modifications or innovations in splenectomy technique are recorded. The selection for the site of the skin incision and laparotomy varied. One method of entry into the peritoneal cavity was between the last two ribs on the left flank (Taliaferro, 1948); Terzian, 1946; Jordan and Robeson, 1942). A slightly different approach to laparotomy was made by Causey (1939) and Al-Dabagh (1960). Both of these researchers made incisions on the left flank, which paralleled the last rib and continued posteriorly along the median line of the abdomen. A small incision through the skin and lateral body wall, on the right side, was made by Toryu (1930) in his work with the carrier-pigeon. Still another variation to expose the spleen was performed by Corradetti and Verolini (1958). These men made a small incision through the body wall, which corresponded to the greater curvature of the stomach, to gain access to this organ.

Upon entry into the peritoneal cavity, several methods have been presented to expose the spleen for removal. Taliaferro (1948) placed small retractors, designed for caponization, between the last two ribs to better

expose the peritoneal cavity. Once inside this cavity, the gizzard and intestines could be pushed aside to reveal the spleen (Causey, 1939; Terzian, 1946; Al-Dabagh, 1960). Taliaferro (1948) and Toryu (1930) revealed the spleen by gently lifting it outside of the body cavity with a hemostat or forceps. Corradetti and Verolini (1958), after cutting the connective tissue which passes from the stomach to the intestine, introduced a self-retaining retractor beneath the stomach to pull it up and to the right. The retractor was then replaced with a Wecker's spatula. This procedure revealed the cavity behind the stomach in which the spleen may be found attached to the dorsal wall of this cavity. The anterior-inferior pole of the canary spleen was easily accessible but the posterior-superior pole must then be pulled down and forward with a Weber's arch to effectuate ligation.

With the spleen uncovered, by the various manipulations described, this organ could then be removed by several techniques. The most common method for removal of the avian spleen was by the ligation of splenic arteries and veins, and cutting of the connective tissue and blood vessels distal to the knot. Toryu (1930), Causey (1939), Terzian (1946), and Al-Dabagh (1960) all used this method for spleen ablation. Jordan and Robeson (1942) utilized cauterization as a successful method of spleen removal. After previous failures to ligate splenic blood vessels prior to spleen recision, these co-workers burned the organ with a blunt steel probe to completely seal off the blood vessels. Corradetti and Verolini (1958) utilized a most unique method for splenectomy of canaries. With the spleen exposed, they carefully tied-off the splenic blood vessels and connective tissue adjoining the spleen. At this point, the spleen capsule was cut longitudinally with a small cataract bistoury (surgical knife), and

the pulp was removed by scrubbing the internal surface of the capsule with a small curette. This technique proved to be less perilous than recision of the spleen with a bistoury or with scissors. From a personal letter of correspondence with Dr. Corradetti, it was learned that the technique described was used only with canaries or other birds of comparable size. For chickens or pigeons he preferred the method of asporting the whole spleen without opening the spleen capsule.

After minor bleeding from spleen removal had ceased, the incisions were closed with sutures. Taliaferro (1948) was able to close the incision very neatly by using the ribs as supports. After removal of the retractor, the ribs could be returned to their natural position and tied together. Suturing the skin was then a simple procedure.

Post-operative techniques were standard procedure for some individuals. For Taliaferro (1948), this consisted of simply keeping the chickens warm. Al-Dabagh (1960) placed chicks in an incubator for several hours after sterilizing and bandaging the site of operation. Mucous secretions were removed from the nostrils of chicks to prevent the possibility of suffocation. Manwell et al. (1957) also found it necessary to remove the mucous from ducklings, for the first few hours following surgery, to prevent accumulation of mucous in the trachea. No reference to post-operative care was mentioned for the remainder of avian splenectomies previously described.

MATERIALS AND METHODS

General Considerations

The investigation considered in this paper was carried out during March, 1970, to January, 1971. The experimental hosts chosen were common pigeons (Columba livia). A prime consideration for this choice was the availability of the bird. Since no fall migration occurs in this particular species, the presence of these individuals, within their natural habitat, could be assured at any time throughout the year. All specimens utilized in this study were taken from a large aggregation of pigeons occurring in downtown Mattoon, Illinois. A vacant four-story hotel harboured the main body of the population.

Pigeons collected at the hotel were usually netted with a long handled aluminum, eighteen-inch net of the type used in landing fish. While collecting pigeons, a face mask with an air filter was worn to help prevent contraction of pigeon-spread respiratory diseases. During the daylight hours, flushed birds could be snared as they flew through the hallways. Pigeons were also trapped after dark, while roosting in rooms within the hotel, with the aid of a sealed-beam lantern. Birds confused by the bright light shining in their eyes, could be approached directly and picked up without using a net. A canvas bag was used for immediate storage of a pigeon upon capture because of its ease of manuverability while pursuing other pigeons. After the bag contained several animals, they were then transferred to portable wire cages for removal to the laboratory where

larger and more permanent quarters were located. Each pigeon was tagged with a numbered plastic leg-band.

The permanent quarters were designed to house thirty or forty pigeons adequately. The dimensions of this structure were approximately eight feet cubed. The cage was constructed of a wood frame covered with chicken wire, and contained within an unheated but weatherproof and verminproof enclosure.

The pigeons were fed a commercial feed which was advertised as a balanced nutritional source. Water was constantly available.

Experimental animals were collected, at random, from their natural habitat. These birds were grossly examined for ectoparasites, wounds, or any manifestation of abnormality. After removal to the laboratory they were monitored, via blood smears, for five days prior to any experimental manipulation for evidence of blood parasites. Positive findings for any of the above were noted and only apparently healthy birds, negative for blood parasites, were utilized experimentally.

Records were maintained of pigeons exhibiting patent infections of hemal parasites upon capture. Birds selected, according to stated criteria, for experimentation were divided into three groups. One group was subjected to splenectomy, another to sham-splenectomy, and the third served as non-incised controls.

Blood smears were obtained at daily intervals, for all individuals in the three groups, during a period beginning seven days before operative procedures, through twenty-one days after, or until death of the individual. Blood smears and spleen impression smears were stained in Giemsa and examined for hemal parasites.

In light of the above procedures, one may pursue a logical line of thought which may reveal the value of splenectomy as a means of detecting

latent malarial infections in pigeons. If a significant number of birds in the splenectomized group experienced relapse, but not a significant number in the sham-operated or non-incised groups relapsed, then one may conclude, logically, that removal of the spleen is related to relapse. If a significant number of birds in the splenectomized group and the sham-operated group experienced relapse but not a significant number in the non-incised group, then one may conclude, logically, that surgical trauma (experience) was in some way related to relapse. One may conclude that relapse was not related to spleen removal or surgical experience if a significant number of pigeons in all groups experienced relapse. If no relapse occurs in any of the experimental groups, then either no infection was present or relapse is related to phenomena not considered in the experiment.

Technical Procedures

Body weights, blood smears, and hematocrits were obtained for all birds prior to operation. An operating board with restraining devices was employed. The board consisted of three-quarter inch plywood, approximately a foot and one-half square. Heavy rubber bands were placed around both legs of the bird just above the feet and around the base of both wings. Care was taken not to occlude circulation. The pigeon was stretched in a diagonal fashion, with the rubber bands around the legs and those around the wings pulling in opposite directions. The birds right wing was reflected dorsally and retained by a rubber strap. A small porous cloth bag was placed over the head of the pigeon to cover the eyes and to minimize excessive movement by the bird. The right flank was adequately exposed by restraining the individual in the above manner. Feathers were plucked from the site of the operation. The feathers immediately surrounding the plucked area were soaked with Zephiran, a surgical disinfectant. No anesthetic was utilized since

pigeons are relatively insensitive in the area of incision and appeared to experience very little pain during operations. Alcohol was used to cleanse and sterilize the skin at the incision site. The location of the incision into the peritoneal cavity was between the last two ribs of the pigeons right side. This location could be determined by palpation of the right flank in the area of the vertebral ribs. The incision was made in a dorso-ventral direction and was usually about two centimeters long, depending on the size of the individual.

The plucked and sterilized skin was stretched caudally before it was opened. The stretching of the skin to one side prevented having a wound in the skin directly above the opening made through the musculature into the peritoneal cavity. After the skin was stretched and lifted, it was then opened from the level of the last dorsal rib. With the skin opened the connective tissue was cleaned away to better expose underlying muscles and nerves. The anterior border of the sartorius muscle was lifted and pulled caudally with a small hand retractor to expose the last two ribs. One or two superficial nerves could be seen traversing the intercostal muscles. These nerve(s) were carefully pulled aside before an incision into the intercostal muscles was made. The belly of the external oblique muscle often could be spared the scapel, since it consisted primarily of aponeurosis at the level of the vertebral ribs. However, it was sometimes necessary to cut six or seven millimeters into the belly of this muscle to obtain adequate retraction of the incision. This technique was necessary for smaller pigeons. The internal oblique was usually spared damage, since the belly of this muscle inserts primarily on the posterior surface of the last rib. Fleshy fibers of the internal oblique also extend downward onto part of the sternal portion of this last rib (George and Berger, 1966).

The transversus abdominis, the deepest of the three flat abdominal muscles, was spared serious damage because of the particular choice of entry into the body cavity.

The parietal peritoneum lines the peritoneal cavity and must be opened carefully to prevent damage of underlying organs. Once the peritoneum was penetrated the ribs could then be retracted. The mesentery which supports the larger right lobe of the liver was cut to facilitate manipulation of this organ. The spleen may be seen lying cradled between the lateral margin of the right lobe of the liver and the intestine. The organ is encompassed by visceral peritoneum and this must be opened to better expose the spleen and facilitate its removal. The pigeon spleen is elongate with one end pointing in a ventral-posterior direction while the opposite end is aimed in a dorsal-craniad direction.

Hyman (1947) described the blood supply of this elongate organ as coming into the peritoneal cavity from the aorta. At the entrance to this cavity the large coeliac artery arises from the aorta. In the pigeon, the coeliac runs posteriorly along the proventriculus to which it branches. This artery then branches to the small left gastric artery, which branches to the left side and edge of the gizzard. The coeliac artery next passes by the spleen, to which it provides a varying number of small splenic arteries; and just beyond the spleen the coeliac gives rise to a hepatoduodenal branch.

With the above description in mind, the posterior end of the spleen, along with the coeliac artery, was lifted with a dull probe, and a hole made in the underlying mesentery. This hole was beneath the spleen and adjacent coeliac, and just posterior to the left gastric branch of this artery. Two non-absorbable silk sutures were passed through the hole in the mesentery and out of the body cavity of the pigeon through the incision.

One of these sutures was slipped around the coeliac artery at the anterior end of the spleen and securely tied. The second suture was then manipulated around this blood vessel at the posterior end of the spleen and ligated anterior to the hepatoduodenal branch of the coeliac artery. The blood supply to the liver (hepatic artery) and duodenum was admittedly curtailed by this surgical technique. However, pigeons can survive this somewhat drastic procedure, because the liver and duodenum may be supplied by branches of the anterior mesenteric and the left gastric arteries. With the blood supply to the spleen shut off, the separation of the spleen from the closely connected coeliac artery and its splenic branches was initiated. The coeliac artery was cut in half, midway between both of the ligatures. The spleen was then excised by cutting through connective tissue and the small splenic arteries between the spleen proper and the coeliac artery.

After the spleen had been removed, the cut edges of the coeliac artery were cauterized. Any splenic tissue remaining attached to this vessel was also cauterized to prevent possible spleen regeneration. The incision was closed by drawing the last two ribs together with sutures. The external oblique was also sutured when necessary. Wound clips were utilized to seal the skin incision after the wound area was saturated with a diluted Zephiran solution.

Post-operative care consisted of placing the splenectomized pigeons in an isolation cage warmed by an electric light bulb as an aid in prevention of shock. Food and water were constantly available. The duration of this care ranged from one to three days depending on recovery rate of individual birds.

Hematocrit and body weights were recorded for all animals before, during, and after experiments. Spleen weight, volume, and dimensions were determined,

as soon as possible, following splenectomy. The weight of the spleen was measured to the nearest one-hundredth of a gram. Spleen volume was determined by the amount of water that this organ would displace in a graduated cylinder. The length of the spleen was measured to the nearest millimeter. Spleen color was observed and any divergence from typical spleen color was noted. After the peritoneal cavity and the gonads were exposed, pigeons were sexed.

The same procedures as used for splenectomy were incorporated into the surgical technique for sham-operated pigeons. The laparotomy phase was identical for both surgical procedures. The spleen was exposed in sham-operated pigeons by the technique described above. In sham-operations, the spleen was left connected and intact. The incision was closed as outlined for splenectomies. Post-operative care was the same for sham-operated pigeons and splenectomized pigeons.

Blood smears were routinely obtained by the toe puncture method. Occasionally blood smears were taken from a recently expired bird by opening the abdominal cavity and inserting the needle of a heparinized syringe upward into the heart or into a major artery and drawing blood.

Spleen impression smears were made from each splenectomized pigeon. Impression smears of liver, lung and brain were made during autopsies. Impressions were made by carefully slicing completely through the particular tissue and placing the sliced edge of this tissue against a clean microscope slide. All blood and impression smears were stained in Giemsa's according to methods employed by Garnham (1966).

Tissues used for histological sections were fixed with Zenker's fluid. Chromatization, washing, dehydration, clearing and embedding in paraffin, followed the methods suggested by Humason (1962).

All stained blood smears as well as impression smears were examined by use of an American Optical binocular microscope with 10x oculars and 100x oil immersion objectives. A minimum of thirty fields or 3,000 red blood cells were examined per blood smear. At least thirty minutes per slide were spent examining impression smears since each slide contained several impressions. Parasite counts were made by the procedure described by Gingrich (1932). Photomicrographs of stained material were taken by a professional photographer.

RESULTS AND DISCUSSION

Blood Smears and Impression Smears

Microgametocytes and macrogametocytes generally occur in circulating erythrocytes of the avian host and are easily demonstrated by blood smears during the patent phase of Haemoproteus infections (Plate I). A mature macrogametocyte can be seen wrapped around the nucleus giving rise to an elongate and "sausage-shaped" organism. The cytoplasm is stained a deep blue color and contains fourteen or more small dark pigment granules. The nucleus may be seen as a small pink area within the blue cytoplasm of the gametocyte. A microgametocyte may be distinguished from a macrogametocyte by a light pink coloration, larger size and fewer number (6-8) of pigment granules, and lighter and more diffuse nucleus. The gametocytes of Haemoproteus in pigeons examined compare morphologically to the description of H. columbae given by Levine (1961) and Garnham (1966). This species is known to occur most frequently in pigeons, according to Mohammed (1958). Therefore, it is reasonable to assume that Haemoproteus columbae is the parasite demonstrated above.

From August through December, eighty pigeons were collected and of these birds, 8.8 per cent (Table 1) exhibited Haemoproteus infections at the time of capture, or became patent sometime during the period in which blood was routinely monitored. This may be compared to an incidence of 27.8 per cent infection, as reported by Fox (1968), on the same population of pigeons. The difference in incidence of infection does not necessarily indicate a decrease of 19.0 per cent, over the three year period, since no

Table 1

INCIDENCE OF *HAEMOPROTEUS COLUMBAE* IN PIGEONS (*COLUMBA LIVIA*) COLLECTED IN COLES COUNTY, ILLINOIS

Date Collected	No. Collected	Positive	Negative	% Positive
8-27-70	16	3	13	18.8
10-15-70	3	0	3	0.0
11-05-70	30	0	30	0.0
11-09-70	18	1	17	5.6
12-15-70	13	3	10	23.1
Totals:	80	7	73	8.8%

specific information concerning the month(s) in which pigeons were collected was mentioned by Fox. An increased incidence of avian hemal infections may occur during the warmer months when the insect vectors are present. Surges in the incidence of Haemoproteus infection are possible at this time not only because of the presence of vectors but also because of susceptible juveniles from recent spring broods. In the month of August, 18.8 per cent of the pigeons collected harboured infections of H. columbae (Table 1). This relatively high incidence is perhaps attributable to the availability of greater numbers of hippoboscid flies and other insect vectors during August in Illinois. No gametocytes could be demonstrated in October and an incidence of only 2.1 per cent was recorded during the month of November. It is probable that the observable decrease in incidence of H. columbae is directly related to the effect of unfavorable climatic conditions on some of the ectoparasitic vectors of Haemoproteus. These vectors should become less prevalent with the arrival of colder months in Illinois. In December, a rate of infection of 23.1 per cent was recorded (Table 1). Relapses of latent infections of Haemoproteus are commonly known to take place in pigeons and is thought to be associated with decline of host immunity. This phenomenon is the probable explanation for individuals exhibiting patent infections during this month, since recurrences of gametocytaemia occur at irregular intervals in the course of infection with H. columbae (Coatney, 1933).

Impression smears of spleen, liver, brain and lung were obtained from twenty-four individuals and 16.6 per cent of these demonstrated the presence of gametocytes of Haemoproteus. However, these gametocytes were located within erythrocytes and probably were in transit through the organ's circulation at the time in which samples were taken. The exoerythrocytic stages of H. columbae are largely confined to endothelial cells in the small capillaries of the lungs; schizonts are less frequently found in the liver and

spleen. Impression smears of lung, liver, spleen, and brain did not provide any evidence of exoerythrocytic schizogony of Haemoproteus in pigeons examined during the present investigation.

The overall incidence of 8.8 per cent infection, as observed in this study, is somewhat low for Haemoproteus infections when compared with other reports on the incidence of this avian parasite. Levine (1961) has listed some representative studies of the incidence of this well known hemal parasite: 100 per cent of 28 pigeons in midwestern United States, 82 per cent of 101 pigeons in the Honolulu zoo, 58 per cent of 159 pigeons in Brazil, and 100 per cent of 75 pigeons examined in Cairo, Egypt. Fox (1968) reported an incidence of 27.8 per cent for 36 pigeons collected in Central Illinois. Levine also cited the incidence of H. columbae for another columbiform bird, the mourning dove (Zenaidura macroura carolinesis), occurring in Illinois: 48 per cent of 188 mourning doves in one study, and 30 per cent of 392 immature and 43 per cent of 72 adult mourning doves in a later survey. All of the infection rates indicated above, are admittedly much higher than the 8.8 per cent recorded in the present investigation. However, certain factors, such as climate and availability of vectors, must be considered when comparing the results of the various surveys mentioned above. The 8.8 per cent incidence observed is expectedly lower, since the majority of pigeons were collected when suitable vectors were unlikely to be present in great numbers. Many infections had declined into a latent phase and were not detectable by ordinary blood smear techniques.

An observed incidence of 20.8 per cent was recorded for organisms identified as Atoxoplasma (Table 2). Identification was based largely upon the criteria for distinguishing stages of Atoxoplasma, as described by Lainson (1959).

Table 2

INCIDENCE OF *ATOXOPLASMA* FROM SPLEEN IMPRESSIONS IN PIGEONS (*COLUMBA LIVIA*), COLLECTED IN COLES COUNTY, ILLINOIS

Date Collected	No. Examined	Positive	Negative	% Positive
8-27-70	16	5	11	31.9
10-15-70	3	0	3	0.0
12-15-70	5	0	5	0.0
Totals:	24	5	19	20.8%

While infected monocytes and lymphocytes do occur in peripheral circulation, none were noticed during the present study, with one possible exception. A blood smear from a single pigeon (188) contained a monocyte with an inclusion suggestive of Atoxoplasma. The host-cell nucleus was indented by this inclusion body. The nucleus of the intraleucocytic body was not visible, but the size and shape of the structure were appropriate for the infective agent. No other distinguishing characteristics suggesting Atoxoplasma could be recognized.

All of the organisms definitely identified as Atoxoplasma were located in the spleen of pigeons examined. However, other tissues are known to harbour these parasites (liver, lung, kidney, and bone marrow). In spleen impressions Atoxoplasma was found to lie within a vacuole in the cytoplasm of a monocyte or large lymphocyte and often indented the host-cell nucleus in a distinctive manner (Plate II). In dried, stained films the outlines of the parasite were seen with difficulty, and the nucleus was poorly differentiated, appearing as a diffuse, pinkish haze in a delicate blue or almost colorless cytoplasm. This shortcoming was probably due to the weak staining reaction which is characteristic of this organism when stained in Giemsa.

The average size for parasites observed in pigeon spleens was 6.1 x 3.4 microns, with a range of from 2.4 x 2.1 to 8.4 x 7.8 microns. Lainson (1959), in blood films from sparrows, reported the average size of the infective agents as being 5.0 x 3.0 microns, with a range of from 3.6 x 1.8 to 5.4 x 3.6 microns. While Lainson measured young "sporozoites" (trophozoites) and the "sporozoites" circulating in the blood of sparrows, none of the latter (intercellular) parasites were seen in the peripheral blood of pigeons examined during the present study. Trophozoites were measured from spleen

impressions of pigeons. It is possible that additional stages other than trophozoites were inadvertently measured, and this may account for the wider range in size of parasites observed. Certain morphological characteristics of Atoxoplasma, such as size, may conceivably be affected by differences in the species of host parasitized. Indeed, this is the case for Atoxoplasma from several species of South Pacific birds (Silvereyes), in which the parasites have a size range of from 4.9 x 2.3 to 11.1 x 6.4, according to Laird (1959).

Lainson (1958) reported that a single organism of Atoxoplasma most frequently parasitizes each host cell, but these organisms may provide one to five parasites to each of these cells. During the present investigation, monocytes have been observed to contain a single parasite for most (10) infected host cells examined. Two monocytes were seen to harbour two organisms while another leucocyte held four inclusions. One lymphoid-macrophage cell was noticed to accommodate five parasites (Plate III); three of these appear to be located within or under the host-cell nucleus and two individuals can be seen to cause a characteristic notching of this nucleus. The range of one to five parasites per host cell was precisely within the normal limits described by Lainson.

While Atoxoplasma has been reported chiefly for passerine species, there is no reason to believe that this parasite does not have a wider range of avian hosts, specifically the Columbiformes. Stages of schizogony were not definitely disclosed in infected pigeons. Intensive schizogony is known to occur early in the spring for sparrows and this asexual cycle gradually declines by August and September. If one assumes a somewhat similar cycle for Atoxoplasma in pigeons, it may be assumed that evidence of schizogony in August and September would be less apparent. Since collection of pigeons did not

commence before the last few days of August, it becomes less difficult to explain the lack of schizogonic stages in the infected pigeons examined. Lainson (1959) admittedly had much trouble in locating sporogonic stages in very heavy infections of over 150 sparrows, but these forms were ultimately found in one young bird.

All infections identified as Atoxoplasma were extremely light, with only one to four cells in spleen impressions demonstrating parasites. It is therefore understandable that the chances for finding sporogonic or other stages in the life cycle of Atoxoplasma by the techniques employed, were remote.

The availability of the vector must be considered when natural populations of birds are surveyed for malarial parasites. Mosquitoes are normally active only during warmer weather in the temperate climates. If these vectors are absent, then the pigeon host may be expected to harbour only latent malarial infections as avian Plasmodium does not have an extended patent period (10-20 days). Blood smears can demonstrate parasites only during the first three weeks after infection by an appropriate vector. This factor undoubtedly affected the chances of finding patent infections of avian Plasmodium by ordinary blood smear methods. In fact, no acute infections were observed by blood smear survey. However, three questionable infections were encountered. Two of these were parasitized erythrocytes which contained an inclusion that was suggestive of a plasmodial trophozoite with red nucleus and pale blue cytoplasm. No pigment granules were observed for either of these erythrocytic inclusions. The third questionable infection was an apparently unpigmented schizont located within a monocyte in the peripheral circulation. The organism contained fourteen visible red nuclei, but the cytoplasm did not stain the characteristic blue

and appeared white against the surrounding blue cytoplasm of the host cell. The appearance of phanerozoites and unpigmented schizonts in the blood, although rare, are possible. Becker (1961) observed an exoerythrocytic stage of Plasmodium relictum with sixteen nuclei in a blood smear drawn by toe puncture from a common pigeon. Manwell (1940) also stated that he saw a typical unpigmented schizont of P. circumflexum within a monocyte in the circulating blood. If all three of these questionable malarial organisms are, indeed, plasmodial parasites then an incidence of 3.8 per cent for eighty pigeons examined by the routine blood smear method, would hold. (Table 3). Impression smears of brain, spleen, lung, and liver provided no evidence of the presence of Plasmodium in pigeons studied.

The results of blood smears and impression smears collectively provided evidence of three genera of hemal parasites, occurring under natural conditions, in the common pigeon (Table 4). An incidence of 8.8 per cent was observed by blood smear methods for Haemoproteus, while a 3.8 per cent (?) occurrence was similarly noticed for Plasmodium. The combined incidence of Haemoproteus and Plasmodium, for the present study, is 12.6 per cent for eighty pigeons. Farmer (1960) reported a comparable incidence of 11.3 per cent as the combined rates of Haemoproteus and Plasmodium in 451 common pigeons (Columba livia) from Iowa. The third genus of hemal parasites, demonstrated in pigeons studied, was Atoxoplasma. Impression smears of the spleen were examined for twenty-four pigeons. Five of these birds were reported to contain organisms identified as Atoxoplasma for an incidence of infection of 20.8 per cent.

Effect of Splenectomy on Relapse

Pigeon number 121, having no demonstrable parasitemia before being splenectomized, did show a .05 per cent parasitemia at twenty-four hours

Table 3

INCIDENCE OF *PLASMODIUM* IN PIGEONS (*COLUMBA LIVIA*)
COLLECTED IN COLES COUNTY, ILLINOIS

Date Collected	No. Collected	Positive	Negative	% Positive
8-27-70	16	1 (?)	15	6.2
10-15-70	3	1 (?)	2	33.3
11-05-70	30	0	30	0.0
11-09-70	18	1 (?)	17	5.6
12-15-70	13	0	13	0.0
Totals:	80	3	77	3.8%

Table 4

RESULTS OF BLOOD SMEARS AND IMPRESSION SMEARS ON PIGEONS (*COLUMBA LIVIA*) COLLECTED IN COLES COUNTY, ILLINOIS

Date Collected	Haemoproteus	Atoxoplasma	Plasmodium
8-27-70	3	5	1 (?)
10-15-70	0	0	1 (?)
11-05-70	0	0	0
11-09-70	1	0	1 (?)
12-15-70	3	0	0
Per Cent Infection:	8.8%	20.8%	3.8%

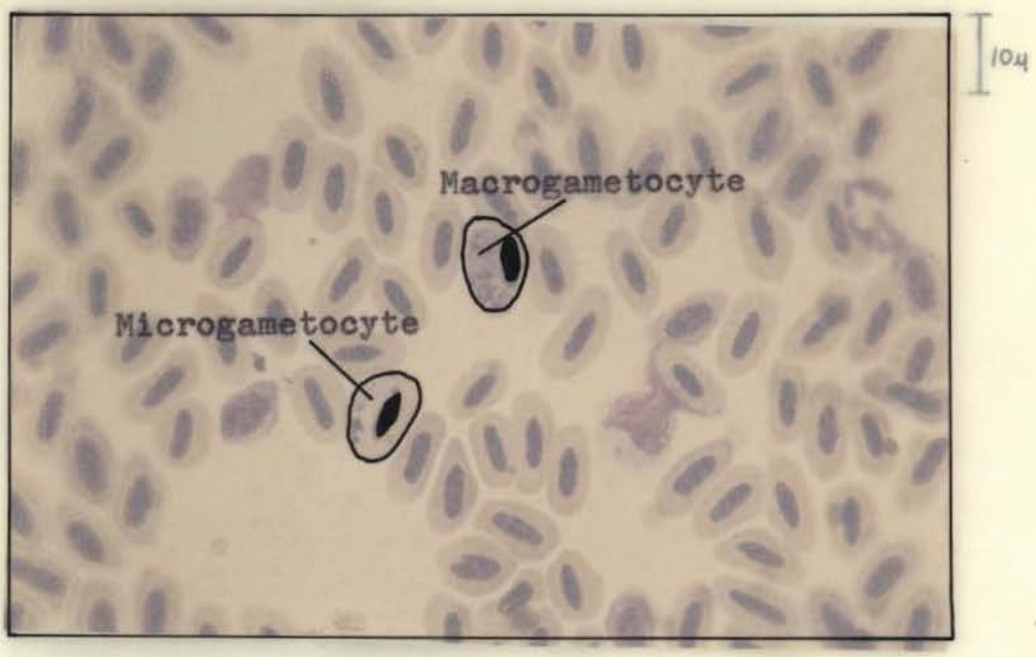
Plate I

A Macrogametocyte and Microgametocyte of *Haemoproteus columbae* in the Blood of a Pigeon (184) (*Columba livia*) Captured in Coles County, Illinois

Plate II

A Trophozoite of *Atoxoplasma* in a Spleen Impression from a Pigeon (178) (*Columba livia*) Captured in Coles County, Illinois

Plate I



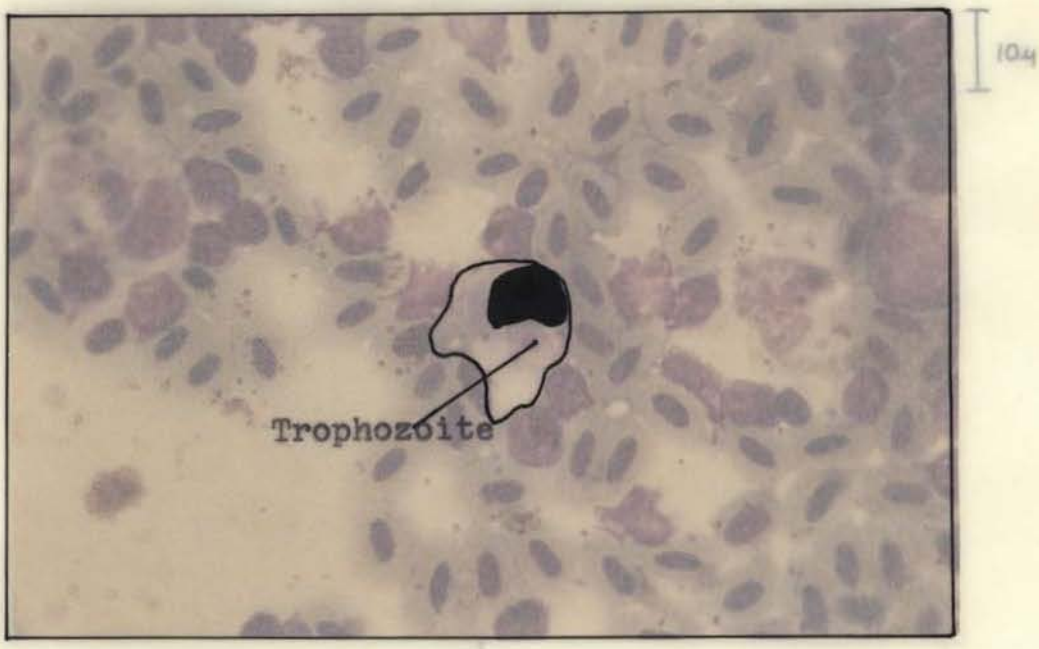
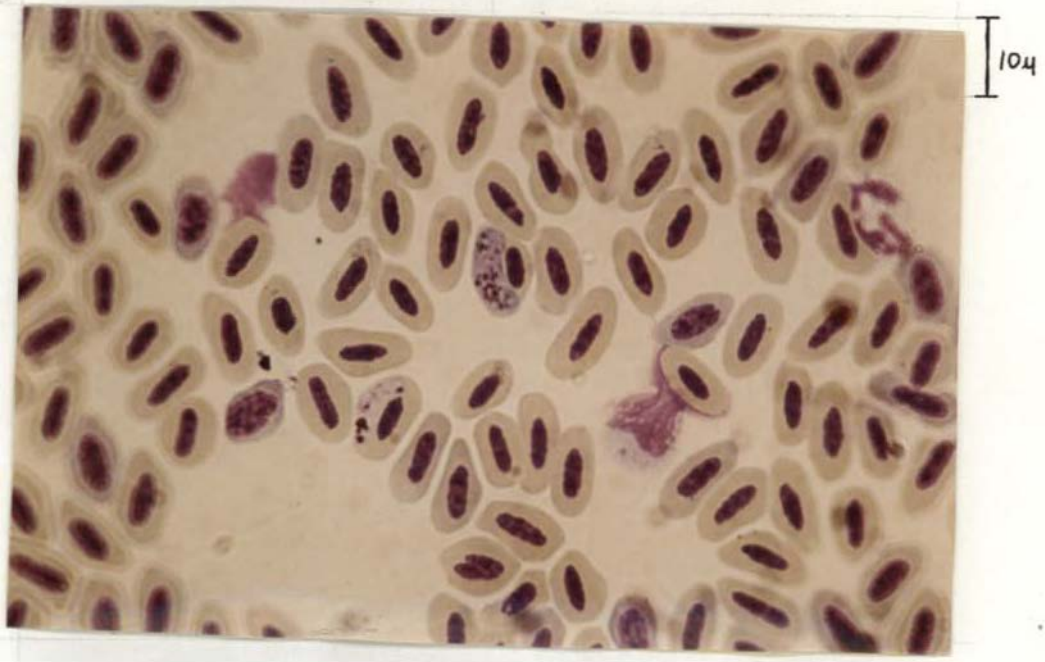


Plate II

Plate I



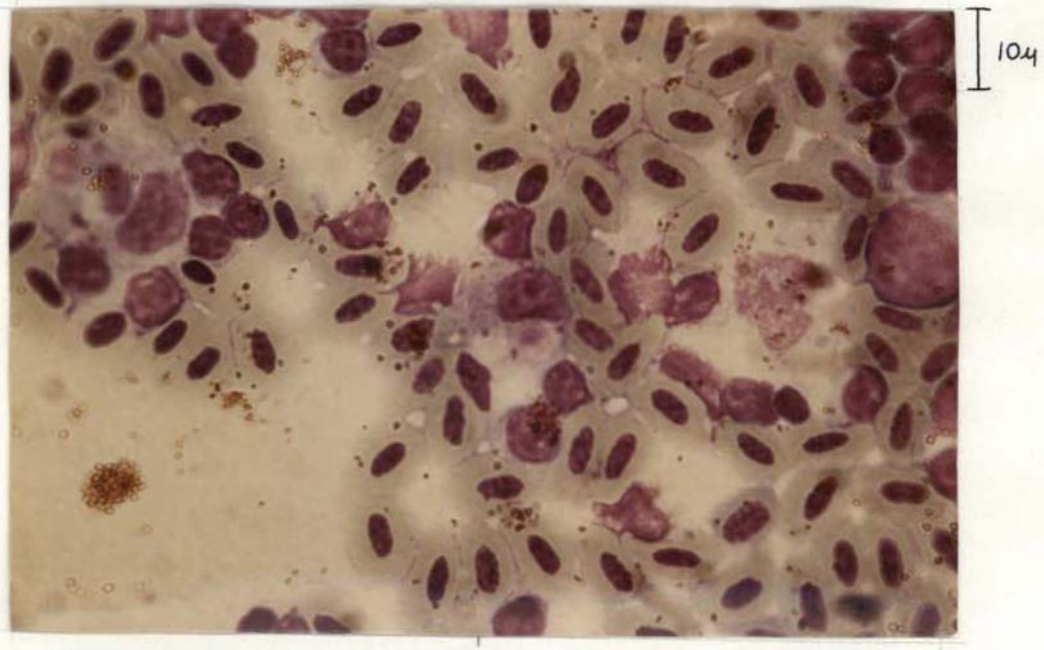


Plate II

Plate III

Trophozoites of *Atoxoplasma* in a Spleen Impression from a Pigeon (178) (*Columba livia*) Captured in Coles County, Illinois

Plate III

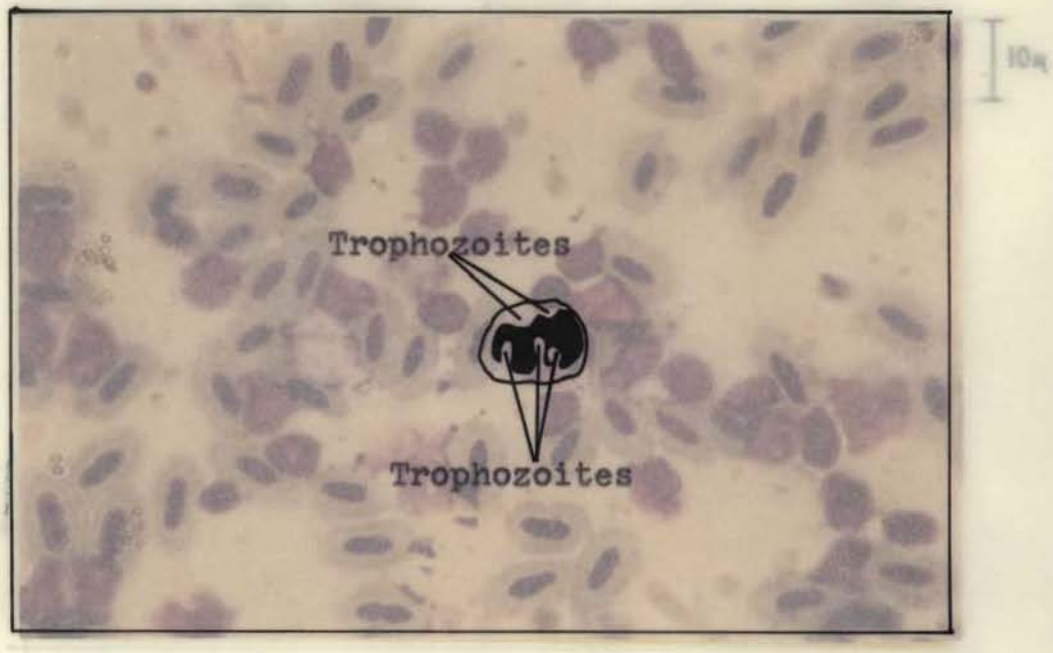
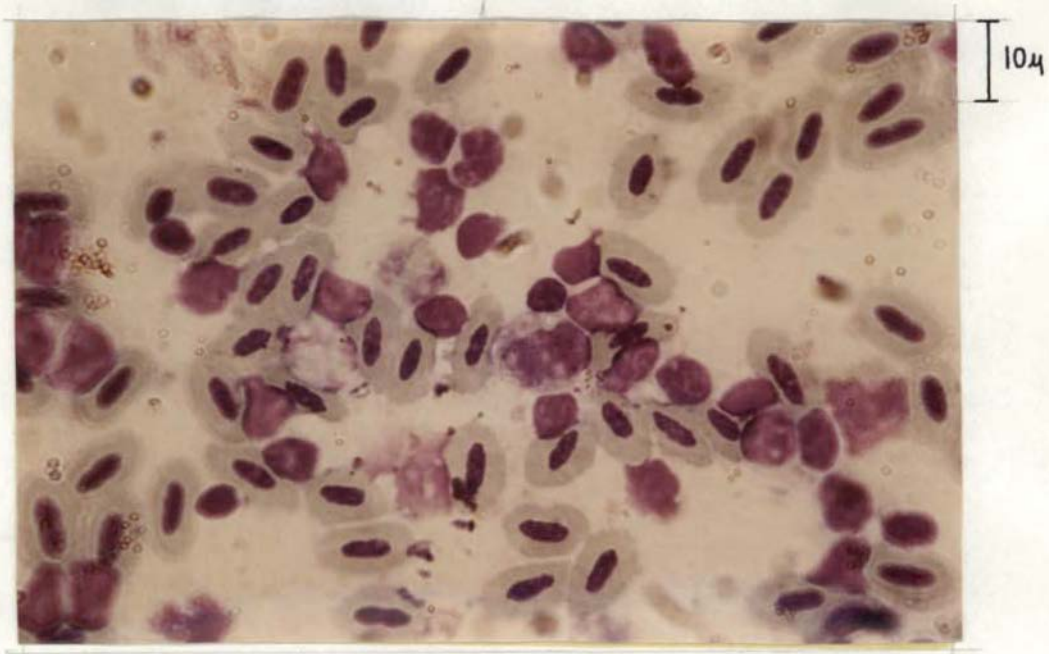


Plate III



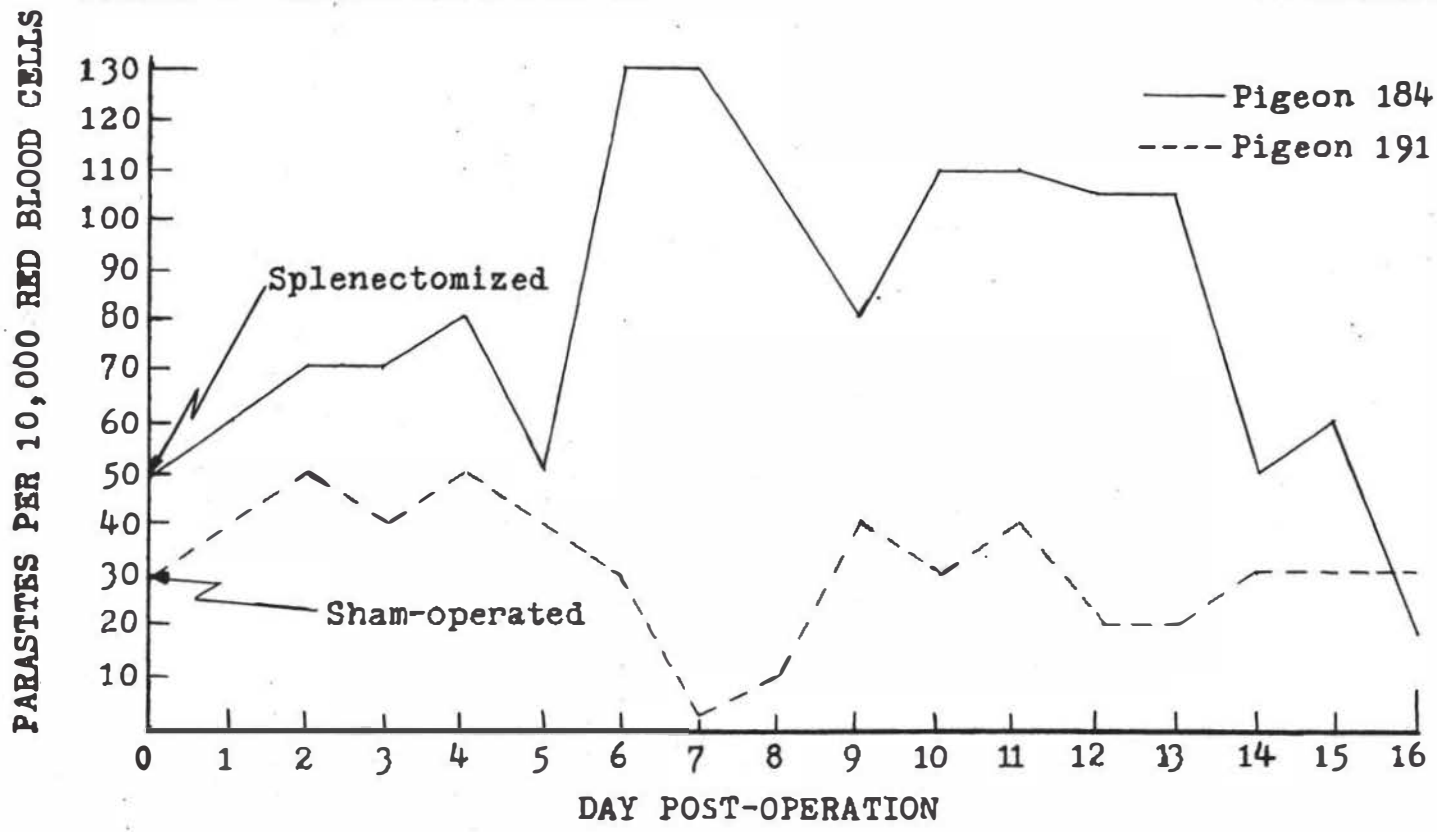
following splenectomy. Mature macrogametocytes were observed in erythrocytes and identified as H. columbae. The patent infection persisted, at a low level, for the following six days at which time the host bird expired. Considering the benign host-parasite relationship of Haemoproteus and pigeons, and the extremely modest parasitemia exhibited in this instance, it is highly unlikely that the sporozoan infection was a cause of death. However, a relapse apparently did occur.

That splenectomy may induce relapse, or at least affect the course of parasitemia in Haemoproteus infections, is suggested by a demonstration using two pigeons harbouring patent H. columbae infections. Pigeon number 184 was splenectomized and pigeon number 191 was subjected to sham-operation. Blood smears were obtained daily for both animals following the surgical procedure. The splenectomized bird showed an increase in parasitemia for seven days following the operation. Parasitemia rose from .30 per cent, at the time of splenectomy, to 1.30 per cent on the sixth day post-operation (Figure 1). On the other hand, pigeon 191 (sham-operated) actually showed a drop in parasitemia, for the first seven post-operative days, from .50 per cent to approximately .02 per cent. Fox (1968) reported a range of parasitemia, for ten pigeons with patent infections of H. columbae, of from .10 per cent to .25 per cent. These ten pigeons exhibited natural infections of Haemoproteus and were collected from the same population as were pigeons for the present investigation. Both birds (184, 191) exceeded the .25 per cent parasitemia observed by Fox, and the splenectomized individual rose to a level of infection of more than five times this figure. It must be stated, however, that pigeons 184 and 191 showed a distinct reversal in parasitemia trends after the seventh day post-operation.

Figure 1. The effect of splenectomy and sham-operation on parasitemia, during patent infections of *Haemoproteus columbae*, in two pigeons (*Columba livia*) from Coles County, Illinois.

Figure 1

EFFECT OF SPLENECTOMY AND SHAM-OPERATION ON PARASITEMIA OF HAEMOPROTEUS



Since the individual that experienced splenectomy (184) demonstrated a much higher parasitemia than the sham-operated pigeon (191), one may suggest that the spleen is, in some way, involved with the host-parasite relationship. The fact that after seven days a sharp decline in parasitemia occurred, in the splenectomized bird, suggests that the host immune mechanism was able to exert an appreciable effect upon the parasite infection in the absence of the spleen. Splenectomy is known to be an experimental method by which an animal may be "immediately" deprived of its acquired immunity and its natural immunity (Garnham, 1963). The host is temporarily left without a defense mechanism against infection. A certain time period must elapse before a compensation, for the great loss in immunity potential, can be made by the splenectomized individual. Eventually, an increase in lymphocytopoiesis will occur in bone marrow, and other lymphoid tissue, to help offset the loss of the spleen. As lymphocytes are once again available, from lymphocytopoiesis, a heteroplastic transformation of these cells into plasma cells can initiate a rise in antibody titer and hence an increased immunity. Lymphocytes are also transformed into macrophages to enhance the phagocytic capabilities of the host organism against parasites. Indeed, this is what apparently took place on the seventh day post-splenectomy for pigeon 184. The sham-operated bird (191) seemed to be relatively unaffected by the surgical trauma and there was no increase in parasitemia. In fact, a decline in parasitemia occurred for seven days post-operation. This observation would indicate that the immune mechanism was not hampered by sham-operation to the degree that the host-parasite relationship was sufficiently altered to cause a surge in the number of Haemoproteus gametocytes. The level of parasitemia of malarial parasites fluctuates with the rise and decline in immunity and this may explain the significant increase of infection on the eighth day following sham-operation in pigeon 191.

From the results of this investigation it would seem that splenectomy is able to induce relapse of latent infections of Haemoproteus, as demonstrated by pigeon 121. The immune mechanism was sufficiently altered to change the host-parasite relationship from a stage of latency to a patent phase of infection. The course of parasitemia, of a patent infection of Haemoproteus (pigeon 184), was also significantly influenced by spleen removal. These observations suggest that the relationship between H. columbae and the pigeons studied was affected by splenectomy and that this procedure can apparently induce relapse. Experimentally induced relapse of H. columbae in the pigeon is relatively unknown. However, such a relapse was observed by Farmer (1970) in several pigeons as a result of spleen ablation performed for other purposes.

No relapse of Plasmodium was observed to occur as a result of splenectomy in the present investigation. Relapse and recrudescence of avian malaria following splenectomy, has been reported for P. cathemerium in canaries (Causey, 1939), P. gallinaceum in chicks (Brumpt, 1936; Terzian, 1946; Taliaferro, 1948; Corradetti, 1955), P. rouxi in canaries (Corradetti and Verolini, 1958), P. juxtanucleare in chicks (Al-Dabagh, 1960), and P. relictum in pigeons (Longenecker, 1968). If the pigeons splenectomized during the present study had harboured latent plasmodial infections, similar to the one reported above by Longenecker, they should have experienced relapse. Such a position may be explainable with a discussion of the specific role played by the spleen in malarial immunity and relapse.

It is known that the spleen has a contribution to the bodily defense against malarial and other parasites of birds (Garnham, 1963), since removal of the spleen during latent infections has resulted in immediate recrudescence for several species of avian Plasmodium mentioned above. This contribution

may be the production of antibody, a direct cellular contribution, or possibly a combination of both of these, according to Longenecker (1968).

Hyperplasia of the lymphoid-macrophage system and heteroplastic transformation of lymphocytes to monocytes and macrophages is the basis of cellular immunity against malarial parasites of all types (Garnham, 1966). An intense lymphocytopoiesis occurs in the nodules of the spleen during malaria, which compensates for the depletion of macrophages and various other lymphoid cells involved in phagocytosis of malarial parasites and pigment.

Reticular cells of the spleen, as well as lymphocytes, are also transformed heteroplastically into plasma cells, which function to produce antibodies. This constitutes the physical basis of humoral immunity to malarial parasites.

Splenectomy immediately deprives an animal of its acquired immunity (premunition) and also its natural immunity. Even though the spleen does not possess as many macrophages as the liver, the spleen is still very important because of the tremendous potential of heteroplastic development of lymphoid elements into macrophages and plasma cells. Therefore, when birds are splenectomized a large part of the lymphoid-macrophage system is lost and these individuals are immunologically deficient until other centers such as the liver and bone marrow can take over. Indeed, Jordan and Robeson (1942) have described the production of lymphoid nodules (lymphocytopoiesis) in the bone marrow directly following splenectomy in the domestic pigeon.

The overall mechanism of avian malarial relapse, as induced experimentally by splenectomy, may be assumed to be similar for most avian species. If this is true, then any of the pigeons splenectomized, which were harbouring latent malarial infections, should have relapsed. Splenectomy did not produce

plasmodial relapse in this experiment, so birds apparently did not harbour this parasite. However, the high mortality rate amoung splenectomized birds certainly decreased the chances for obtaining a relapse from latent infections of Plasmodium.

Of twenty-one pigeons that survived the operation, three died on the same day in which splenectomy took place. Four birds expired within 48 hours after spleen removal and nine animals succumbed during the next five days. The remaining individuals that experienced splenectomy, survived for a period of from five to fourteen weeks depending upon the time when these apparently recovered pigeons were sacrificed for autopsy. However, if relapse of latent infections is to occur, it should take place within seventy-two hours. According to Garnham (1963), splenectomy "immediately" removes natural and acquired immunity. Al-Dabagh (1960), El-Nahal (1966), and others have reported a resurgence of parasitemia within seventy-two hours post-splenectomy for latent infections of Plasmodium.

Effect of Splenectomy on Body Weight

Splenectomized birds declined sharply in body weight for the first five days following the operation (Figure 2, 3). No such decline was evident in non-incised controls weighed during the same period (Figure 2, 3). That weight loss is not a result of spleen removal per se, but more a consequence of surgical trauma, is suggested by the fact that sham-operated pigeons also experienced a weight reduction during the initial post-operative period (Figure 3). Pigeons may normally exhibit a fluctuation in body weight, ranging from ten to twenty grams over a twenty-four hour period. Fluctuation is related to feeding habits and physiology and was taken into account in assessing body weight variation in experimental animals. The more rapid recovery of body weight, following decline

by sham-operated birds, may be attributed to the considerably less surgical trauma experienced by these pigeons as compared to splenectomized individuals. Of course, decreased competition for food and water, as a result of being isolated in separate cages, contributed to experimental error in evaluating weight loss for both sets of operative birds.

Crop smears of thirteen pigeons used in this investigation indicated that approximately 45 per cent of these birds harboured Trichomonas columbae. A high incidence of Trichomonas is not unusual, and this parasite has been found in 69 per cent of captured pigeons, according to Biester and Schwarte (1965). The observation of crop smears taken on post-operative birds showing weight loss, suggests that Trichomonas infection becomes more severe following splenectomy. No line of investigation was initiated to follow up this suggestion.

Effect of Splenectomy on Hematocrit

Sturkie (1965) reported an average hematocrit reading for apparently healthy pigeons of 57.5 per cent. The average hematocrit reading, observed during the present study for non-incised controls, was approximately 62 per cent (Figure 4). This certainly approximates the value for hematocrit as reported by Sturkie, considering the method of determining hematocrit readings is subject to human error. Acclimatization to cold temperatures may partially explain the higher average hematocrit, experienced during this study, as compared with the average value given by Sturkie (1965). The diminution of the hematocrit and plasma volume is known to take place as a feature of acclimatization to high environmental temperatures, according to Sturkie. An increase of hematocrit and plasma volume possibly occurs as a result of acclimatization to low environmental temperatures. Pigeons used during the present investigation were housed in unheated quarters and the outside air

temperature averaged 25° F. It is reasonable that acclimatization to cold weather may be partially responsible for the slightly higher hematocrit readings for pigeons observed in this study.

Splenectomy resulted in a hematocrit decline of approximately 7 per cent while sham-operation led to a 5 per cent decrease in corpuscular volume (Figures 4, 5). Naturally, more blood was lost by spleen removal because of the very nature of the operation. The direct loss of blood, in both surgical procedures, is the most probable explanation for the average decline in hematocrit post-operation. However, the diminution of average hematocrits during the present investigation is considerably less than the reduction experienced by other researchers.

Toryu (1930) reported an immediate decrease in the number of erythrocytes (often 25 per cent or more) in his carrier-pigeons subsequent to splenectomy. From this information, it is evident that the procedure used by Toryu, for spleen extirpation, involved a much greater loss of blood than that encountered in the present study. Erythrocyte decrease is associated with a considerable polychromatophile increase during the first two weeks post-operation, suggesting an increased haemopoiesis (Toryu, 1930). Increased numbers of polychromatophiles (reticulocytes) were sometimes noticeable, during this experiment, in blood smears taken shortly following operations.

Gross Observations on Pigeon Spleens

Several measurements were taken for twenty excised pigeon spleens, during the present study. The weight, length, and volume were determined by methods described earlier. An average weight of 230 milligrams (110-390 mg.) for males (10) and females (10) was observed. There was no correlation between body weight and spleen weight, and sex and spleen weight. Spleen volumes averaged 2 milliliters (1-6 ml.) for the twenty pigeons

Figure 2. Average body weight of four pigeons post-splenectomy compared with average body weight of three non-incised controls.

Figure 3. Average body weight of four pigeons after sham-operation compared with average body weight of three non-incised controls.

Figure 4. Average hematocrit of three pigeons post-splenectomy compared with average hematocrit of three non-incised controls.

Figure 5. Average hematocrit of five pigeons after sham-operation compared with average hematocrit of three non-incised controls.

FIGURE 2

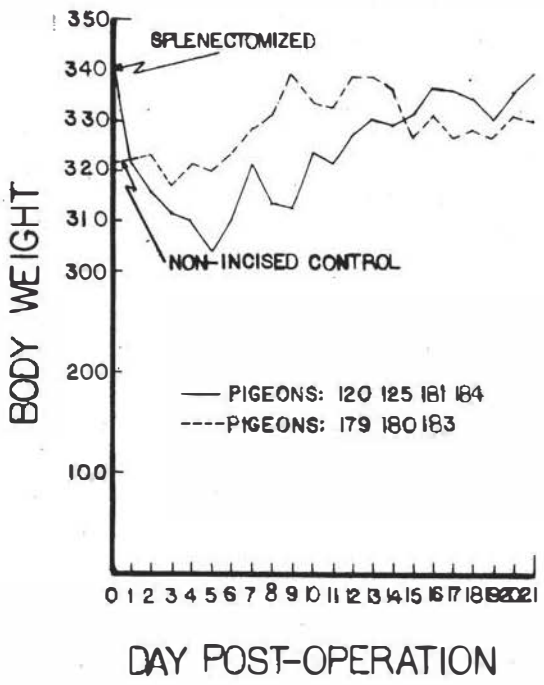


FIGURE 3

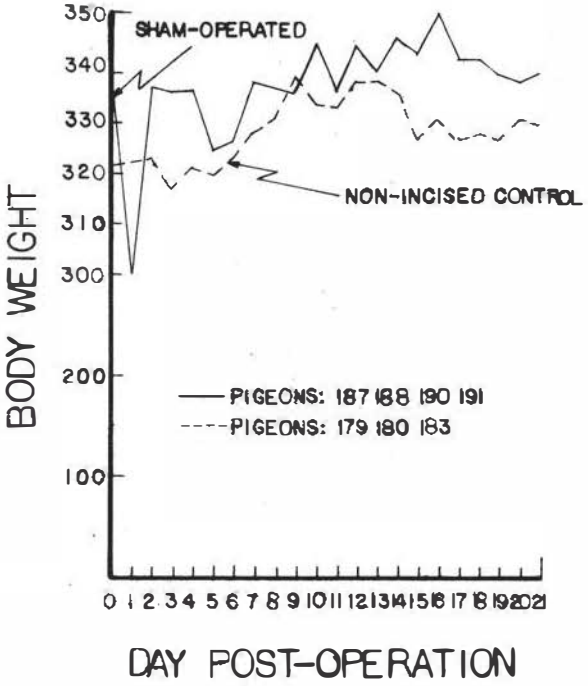


FIGURE 4

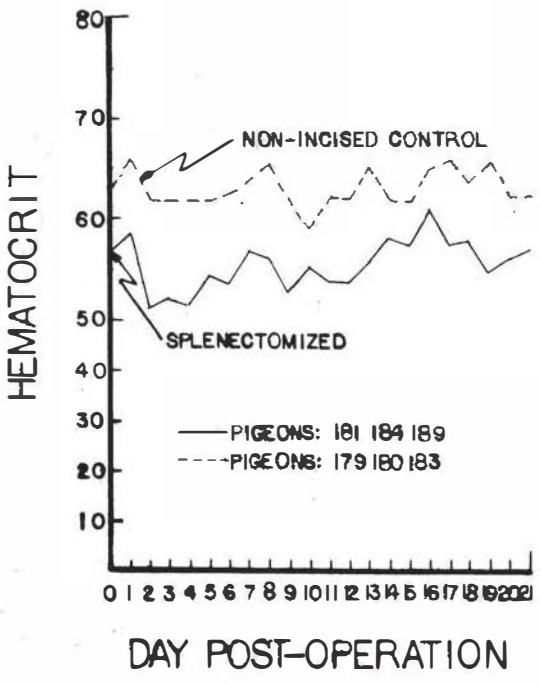
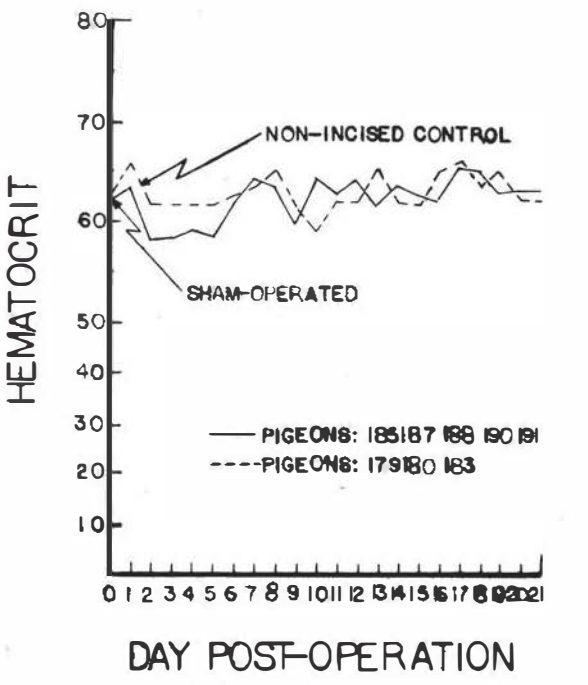


FIGURE 5



examined in the present investigation. The average spleen length, for birds examined, was 11.4 millimeters with a range of 8 to 13 millimeters. Jordan and Robeson (1942) emphasized that the spleen of the domestic pigeon varied considerably in size. These workers reported spleen lengths of 7 to 9 millimeters for adults. Squabs were reported to have spleens averaging 10 millimeters in length. No information concerning the number of spleens measured was given.

Any divergence in "normal" spleen color or size was also noted during this study. Malarial parasites are easily digested by phagocytes in the spleen, but the parasite pigment remains and blackening of this organ is a feature of infection. Pigeon number 184 harboured a patent infection of Haemoproteus columbae at the time of splenectomy, and the spleen was definitely atypical. This organ was not only dark purple in color but was also noticeably oversize. Splenomegaly commonly accomanies malarial infection and, therefore, it is not surprising that this was the largest spleen measured. All infected pigeons had enlarged spleens. However, several birds had enlarged spleens of a "normal" color and showed no evidence of infection.

SUMMARY AND CONCLUSION

1. A survey of naturally occurring hemal parasites of pigeons from Coles County, Illinois was conducted during the fall and winter of 1970. Blood smear and impression smear techniques and splenectomy procedures were the methods utilized for examination of individuals studied.

2. Routine blood smears were employed to disclose patent infections of blood parasites. Eighty pigeons were surveyed by this method and seven patent infections of Haemoproteus columbae were demonstrated for an incidence of 8.8 per cent. Haemosporidian parasites of the genus Plasmodium were identified in three of the eighty birds examined to give an incidence of infection of 3.8 per cent.

3. Impression smears of lungs, liver, spleen, and brain tissue did not provide evidence of exoerythrocytic stages of Haemoproteus or Plasmodium. However, a third genus of sporozoan parasites was located by impression smears of the spleen. Organisms identified as Atoxoplasma inhabited monocytes or large lymphocytes within splenic tissue. These individuals were observed to indent the host cell nucleus in the characteristic manner described for this parasite. An incidence of 20.8 per cent infection, for five of twenty-four pigeon spleens examined, was reported as a result of this line of investigation.

4. Spleen removal was employed in an attempt to induce relapse of latent protozoal infections of pigeons. A relapse of Haemoproteus columbae took place in one pigeon within twenty-four hours post-splenectomy and a patent infection continued for six days, at which time the bird expired. A demonstration of the effect of spleen extirpation on a patent infection

of H. columbae further suggested that the host-parasite relationship between Haemoproteus and the pigeon host may be sufficiently altered by splenectomy to cause an increased parasitemia. No relapse of avian Plasmodium was observable in any of the twenty-four birds subjected to splenectomy, and it was concluded that none of these individuals harboured plasmodial infections.

5. Loss in body weight occurred for pigeons splenectomized (10.3% loss) and sham-operated (10.0% loss). No such loss was evident in non-incised controls. A direct correlation between the degree of surgical trauma and decline in body weight was noticeable since splenectomized individuals required a longer period of time to regain lost weight.

6. Splenectomy resulted in a decline in average hematocrit of approximately 7 per cent while sham-operation led to a 5 per cent decrease in corpuscular volume. The diminution of hematocrit for both surgical procedures, suggests a direct relationship between the amount of blood lost and the degree to which corpuscular volume (hematocrit) declines.

7. Several gross observations were recorded from twenty pigeon spleens. The average length of this organ was found to be 11.4 millimeters (8-13 mm.), while average spleen volume determined was 2.0 milliliters (1-6 ml.).

8. The incidence of 8.8 per cent infection for Haemoproteus columbae in pigeons, during the present study, is much lower than the incidence of 27.8 per cent as reported by Fox (1968). Birds were collected, during the present investigation, at a time when insect vectors for Haemoproteus were not in abundance and many parasitized individuals were in a latent phase of infection. Latent infections are not detectable by ordinary blood smears. Therefore, it is understandable that a comparatively lower incidence of infection was observed. An incidence of 3.8 per cent for Plasmodium approximates the value given by Fox (2.8 per cent) for this hemal parasite.

9. No clear cut conclusion may be drawn from the results of the present study regarding splenectomy as a survey tool. High mortality rate in operative individuals suggests a need for further refinement of the technique. Splenectomy did not cause relapse of Plasmodium in any experimental birds, and the assumption is that this infective agent was not present. However, there is evidence to support the use of splenectomy to disclose hemal parasites in the avian host. Relapse of a latent infection of Haemoproteus columbae, in a splenectomized bird, indicates a practical application of spleen removal to uncover similar infections from natural populations. Blood smears cannot reveal latent infections of H. columbae and other methods, such as isodiagnosis, are unable to readily expose these infections. Therefore, splenectomy procedures may be a useful diagnostic tool where other techniques are inadequate for the detection of H. columbae, as well as Plasmodium, in natural populations of pigeons.

LITERATURE CITED

Abramoff, P., and M. F. La Via. 1970. Biology of the Immune Response. McGraw-Hill Book Company Inc., New York, pages 272-280.

Al-Dabagh, M. A. 1960. The effect of splenectomy on Plasmodium juxtanucleare infections in chicks. Trans. Roy. Soc. Trop. Med. Hyg. 4: 400-405.

Allison, A. C., and G. P. Bush. 1955. Enzyme activity as a function of age in the human erythrocyte. Brit. Jour. Haemat. 1: 291.

Barcroft, J. 1925. The significance of haemoglobin in sub-mammalian forms of life. Physiol. Rev. 5: 596.

Becker, E. R. 1961. Some unfinished investigations of malaria in pigeons. Jour. Protozool. 8(1): 1-7.

Becker, E. R., W. F. Hollander, and W. H. Patillo. 1956. Naturally occurring Plasmodium and Haemoproteus infections in the common pigeon. Jour. Parasit. 42: 474-478.

Ben-Harel, S. 1923. Studies on bird malaria in relation to the mechanism of relapse. Amer. Jour. Hyg. 3: 652-658.

Biester, H. E., and L. H. Schwarte. 1965. Diseases of Poultry. The Iowa State University Press, Ames, Iowa, pages 1110-1145.

Blaustein, A. 1963. The Spleen. McGraw-Hill Book Company Inc., New York, pages 1-44.

Bloom, W., and D. W. Fawcett. 1962. A Textbook of Histology. W. B. Saunders, Philadelphia, pages 303-313.

Brumpt, E. 1936. Receptivite de divers oiseaux domestiques et sauvage au parasite (Plasmodium gallinaceum) du paludisme de la poule domestique. Transmission de cet hematozoaire par le mostique Stegomyia fasciata, C. R. Acad. Sci. 203: 750-753.

Causey, O. R. 1939. The effect of splenectomy on the course of malarial infection in canaries. Amer. Jour. Hyg. 30: 93-99.

Coatney, G. R. 1933. Relapse and associated phenomena in the Haemoproteus infection of the pigeon. Amer. Jour. Hyg. 18: 133-160.

Corradetti, A. 1955. Studies on comparative pathology and immunity in Plasmodium infections of mammals and birds. Trans. Roy. Soc. Trop. Med. Hyg. 49: 311.

Corradetti, A. 1970. Personal communication.

Corradetti, A., and F. Verolini. 1958. Azione della splenectomia nell' infezione da Plasmodium rouxi nei canarini. Riv. Parassit. 19: 21-28.

Crosby, W. H. 1952. The pathologenesis of spherocytes and leptocytes (target cells). Blood 7: 261.

Crosby, W. H. 1959. Normal function of the spleen relative to red blood cells: A review. Blood 14(4): 399-408.

Crosby, W. H., and J. H. Akeroyd. 1952. The limit of haemoglobin synthesis in hereditary hemolytic anemia. Amer. Jour. Med. 13: 273.

Ebert, R. V., and E. A. Stead. 1941. Demonstration that in normal man no reserves of blood are mobilized. Amer. Jour. Med. Sc. 201: 655-664.

El-Nahal. 1966. Fluorescent antibody studies on infection with Plasmodium gallinaceum: Effect of splenectomy during latency on parasitemia and antibody titer. Jour. Parasit. 52: 570-572.

Farmer, J. N. 1960. Some blood parasites from birds in Central Iowa. Iowa Acad. of Sci. 67: 591-597.

Farmer, J. N. 1970. Personal communication.

Fox, E. G. 1968. Studies on blood parasites of birds in Coles County, Illinois. M.S. thesis, Eastern Illinois University.

Fragraeus, A. 1948. The plasma cellular reaction and its relation to the formation of antibodies in vitro. Jour. Immunol. 58: 1.

Gandal, C. P. 1956. Satisfactory general anesthesia in birds. Jour. Amer. Vet. Med. Assoc. 141: 332-334.

Garnham, P. C. C. 1950. Blood parasites of East African vertebrates with a brief description of exoerythrocytic schizogony in Plasmodium pitmani. Parasitology 40: 328-337.

Garnham, P. C. C. 1966. Malarial Parasites and Other Haemosporidia. Blackwell Scientific Publications, Oxford.

Garnham, P. C. C., A. E. Pierce, and I. Roitt. 1963. Immunity to Protozoa. Blackwell Scientific Publications, Oxford, pages 3-69.

George, J. C., and A. J. Berger. 1966. Avian Myology. Academic Press Inc., New York, pages 284-286.

Ginrich, W. 1932. Methods of estimating haemosporidial infections. Jour. Prev. Med. 6: 197-246.

Harmon, I. W., E. Ogden, and S. F. Cook. 1932. The reservoir function of the spleen of fowls. Amer. Jour. Physiol. 100(1): 99-100.

Herman, C., and A. I. Goldfarb. 1939. Temporary infections with Plasmodium circumflexum in splenectomized chicks. Amer. Jour. Trop. Med. 19: 593-598.

Hewitt, T. 1940. Bird Malaria. John Hopkins Press, Baltimore.

Humason, G. L. 1962. Animal Tissue Techniques. W. H. Freeman and Company, San Francisco.

Hyman, L. H. 1962. Comparative Vertebrate Anatomy. University of Chicago Press, pages 351-358.

Jordan, H. E. 1954. The origin and fate of plasmocytes. Anat. Rec. 119: 235.

Jordan, H. E., and J. M. Robeson. 1942. The production of lymphoid nodules in the bone marrow of the domestic pigeon following splenectomy. Amer. Jour. Anat. 71: 181-205.

Krumbhaar, E. B. 1926. Functions of the spleen (Mysterii Plenum Organon) Galen. Physiol. Rev. 6(1): 160-200.

Lainson, R. 1958. Atoxoplasma Garnham, 1950, in an English Sparrow (Passer domesticus domesticus Linn.). Trans. Roy. Soc. Trop. Med. Hyg. 52: 15-16.

Lainson, R. 1959. Atoxoplasma Garnham, 1950, as a synonym for Lankestrella Labbe, 1899. Its life cycle in the English Sparrow (Passer domesticus domesticus, Linn.). Jour. Protozool. 6(4): 360-371.

Laird, M. 1959. Atoxoplasma paddae (Aragao) from several South Pacific Silvereyes (Zosteropidae) and a New Zealand Rail. Jour. Parasit. 45: 47-52.

Levine, N. D. 1961. Protozoan Parasites of Domestic Animals and Man. Burgess Publishing Company, Minneapolis.

Longenecker, M. B. 1968. The influence of the bursa of fabricius, spleen, and thymus on the course of Plasmodium lophurae infection in chickens. Ph.D. dissertation, University of Missouri.

Manwell, R. D. 1929. Relapse in bird malaria. Amer. Jour. Hyg. 9: 308-345.

Manwell, R. D. 1940. Life cycle of Plasmodium relictum var. matutinum. Amer. Jour. Trop. Med. 20: 859-867.

Manwell, R. D. 1968. Introduction to Protozoology. Dover Publications Inc., New York, pages 487-514.

Manwell, R. D., M. L. Weiss, and A. A. Spandorf. 1957. Effects of splenectomy on elongatum and circumflexum malaria in ducks. Exptl. Parasitol. 6: 358-366.

Miller, E. B., K. Singer, and W. Dameshek. 1942. Experimental production of target cells by splenectomy and interference with splenic circulation. Proc. Soc. Exper. Biol. and Med. 49: 42.

Mohammed, A. H. H. 1958. Systematic and Experimental Studies on Protozoal Blood Parasites of Egyptian Birds. Cairo University Press.

Nicol, T. D., L. J. Bilbey, and C. C. Ware. 1958. Effect of splenectomy on the phagocytic activity of the reticulo-endothelial system. Nature 182: 534-535.

Rous, P., and O. H. Robertson. 1917. The normal fate of erythrocytes. I. The findings in healthy animals. Jour. Exper. Med. 25: 651.

Singer, K., and L. Weisz. 1945. The life cycle of the erythrocyte after splenectomy and the problem of splenic hemolysis and target cell formation. Amer. Jour. Med. Sci. 210: 301.

Stewart, W. B., J. M. Stewart, M. J. Izzo, and L. E. Young. 1950. Age as affecting the osmotic and mechanical fragility of dog erythrocytes tagged with radioactive iron. Jour. Exper. Med. 91: 147.

Sturkie, P. D. 1965. Avian Physiology. Cornell University Press, Ithaca, New York.

Taliaferro, L. 1925. Infection and resistance in bird malaria with special reference to periodicity and rate of reproduction of the parasite. Amer. Jour. Hyg. 5: 742-789.

Taliaferro, W. H. 1948. The role of the spleen and the lymphoid-macrophage system in the quinine treatment of gallinaceum malaria. I. Acquired immunity and phagocytosis. Jour. Infect. Dis. 83: 164-180.

Taliaferro, W. H., and F. E. Kelsey. 1948. The role of the lymphoid-macrophage system in the quinine treatment of gallinaceum malaria. II. Quinine blood levels. Jour. Infect. Dis. 83: 181-199.

Terzian, L. A. 1946. The effect of splenectomy on avian malarial infections. Jour. Inf. Dis. 79: 215-220.

Thiel, Y. 1930. Changes in the blood picture, and in oxygen capacity of the blood haemoglobin of the carrier-pigeon following splenectomy. Sci. Rpt. Tohoku. Imp. Univ. 4th Ser. Biol. 5: 391-402.

Whitmore, E. 1922. The action of light in the production of relapse in malaria. Amer. Jour. Trop. Med. 2: 475-485.